Naval Health Research Center Detachment (Toxicology)

CHARACTERIZATION OF THE ECOTOXICITY OF FIVE BIODEGREDABLE POLYMERS UNDER CONSIDERATION BY NAVAIR FOR USE IN CHAFF-DISPENSING SYSTEMS

TOXDET-01-03

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14. **ABSTRACT** *(Maximum 200 words)*
The accumulation of discarded chaff dispenser styrene piston and endcaps in the environment is a concern of the Department of Defense. Five biodegradable materials are being considered for use in the manufacture of degradable chaff cartridges, pistons, and endcaps. Relative degradability of the materials is being evaluated by measuring total organic carbon (TOC) released by the materials over time in water. Environmental toxicity testing of the dissolution products from the materials is being conducted in 2 terrestrial plant species and 7 species of aquatic organisms. Dissolution products from 4 of the biopolymers were found to be toxic to aquatic organisms with LC50s and LOELs ranging between 1.24 – 731.30 mg of TOC/l. The dissolution products did not inhibit seed germination of *Brassica rappa* or *Lepidium sativum*. Average shoot length of *B. rappa* seedlings treated with BAK2195/CP1000 (90:10) and BIPOL D411GN were 25-40% shorter as compared with negative controls ($p<0.05$). Average shoot length of *L. sativum* seedlings treated with BAK2195/CP1000 (90:10) were 10-20% shorter than controls ($p<0.05$). Information gained from these studies will be used for making decisions on which (if any) of the polymers will be suitable for the construction of biodegradable chaff cartridges, pistons, and endcaps.

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PREFACE

This document contains the results of an investigation of the toxicity of the dissolution products of five biodegradable polymers under consideration for use by the U.S. Navy Naval Air Systems (NAVAIR). The polymers are being considered for use in the construction of "environmentally friendly" chaff dispenser pistons and endcaps. This work was conducted under the direction of CAPT Kenneth R. Still, MSC, USN, Officer-in-Charge Naval Health Research Center Detachment Toxicology (NHRC/TD) and coordinated by LT Cody L. Wilson, MSC, USNR (NHRC/TD). The plant toxicity studies described in this report were conducted by LT Darryl Arfsten (NHRC/TD) in the laboratory of Dr. Barry Spargo, U.S. Naval Research Laboratory. The aquatic toxicity studies were conducted for NHRC/TD by Dr. Dennis Burton at the Wye Research Institute (University of Maryland) under contract by the NHRC/TD. The research was requested and sponsored by Naval Air Warfare Center Aircraft Division under reimbursable Work Unit # 1822.

The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of Defense, the Department of the Navy or the Naval Services at large.

Characterization of the Ecotoxicity of Five Biodegredable Polymers under Consideration by NAVAIR for Use in Chaff-Dispensing Systems

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EXECUTIVE SUMMARY

Background

At least 500 tons of chaff are released annually during training by U.S. forces (USAF 2001) and discarded styrene dispenser pistons and endcaps account for about 95% of the total mass released to the environment (Wilson et al. 2001). In 1999, U.S. Naval Air Systems (NAVAIR) initiated a program to evaluate the toxicity of several biodegradable polymers that could be used in piston and endcap construction. Naval Health Research Center Detachment (Toxicology), Wright-Patterson Air Force Base, OH, was asked to carry out studies to characterize the ecotoxicity of five polymers under consideration for use by NAVAIR for construction of pistons and endcaps.

Methods

Polymers evaluated for their ecotoxicity by NHRC/TD were Polytriticum 2000, BIOPOL D411GN, BAK 402-005, PVA CP1000, and BAK2195/CP1000 (90:10). The stability of the polymers over time was evaluated by placing the materials in deionized or seawater for periods of up to 90 days and measuring the total organic carbon (TOC) content of the water. Toxicity of the materials to aquatic organisms and plants was evaluated by allowing the parent materials to dissolve in water after being stirred continuously over a 24-hour period. At the end of 24 hours, the supernatant (e.g., decant) was separated from remaining parent material and used as the material for toxicity testing. The following tests were conducted with freshwater organisms exposed to each test material: 1) 96-h chronic growth test with the green alga *Selenastrum*

capricornutum; 2) 48-h acute and 7-d chronic survival and reproduction test with the daphnid *Ceriodaphnia dubia*; 3) 96-h acute and 7-d chronic survival and growth test with the fathead minnow *Pimephales promelas*; and 4) 10-d acute and 28-d chronic survival, growth, and reproductive maturation test with the amphipod *Hyalella azteca*. The following tests were conducted with saltwater organisms exposed to each test material: 1) 96-h acute and 7-d chronic survival, growth, and fecundity test with the mysid *Americamysis bahia*; 2) 96-h acute and 7-d chronic survival and growth test with the sheepshead minnow *Cyprinodon variegatus*; and 3) 10-d acute and 28-d chronic survival, growth, and reproduction test with the amphipod *Leptocheirus plumulosus*.

The toxicity of test material decants was also evaluated in two terrestrial plant species *Brassica rappa* or *Lepidium sativum* using standard methods devised by the American Society for Testing and Materials. Seeds of both species were watered with test material decants for 96 hours and grown under constant lighting at 20°C. At the end of the test period, the percentage of emergent seeds was determined and the shoot length of emergent seeds was measured and compared with those of seeds and plants watered with deionized water (e.g., negative controls).

Results

Dissolution studies gave evidence that four out of five polymers partially dissolved in deionized and/or seawater over time while under static conditions. Increasing amounts of TOC were present in vials containing polymers Polytriticum 2000, BAK402-005, PVA CP1000, BAK 2195/CP1000 (90:10) with increasing incubation times. The amounts of TOC measured in vials containing BIOPOL D411GN did not increase with increasing incubation time.

With the exception of BAK2195/CP1000 (90:10), decants of the polymers were found to be toxic to at least one species of aquatic organism selected for toxicity testing. LC50 and LOEC values for these materials ranged between 1.24 – 731.30 mg/L TOC, assuming that above background TOC concentrations were representative of dissolved study materials. Several of the materials were not toxic to many of the selected aquatic organisms at the highest concentrations measured of dissolved study materials A comparison of the study material toxicity values with LC50s and LOECs reported for copper, ammonia, nitrate, sodium chloride, and ethanol suggest that in most cases, these materials are not as toxic as copper, ammonia, or nitrate are to most aquatic organisms, but are slightly more toxic than sodium chloride or ethanol.

There was no consistent evidence that any of the five dissolved polymers had a negative impact on the percentage of emergent *B. rappa* or *L. sativum* seeds as compared with negative controls. Average shoot lengths of emergent *B. rappa* seedlings treated with BIOPOL D411GN or BAK2195/CP1000 (90:10) were reduced 10-20% as compared with the average shoot length for negative controls ($p<0.05$). Average shoot length of *L. sativum* seedlings treated with BAK2195/CP1000 (90:10) were 10-20% shorter than controls ($p<0.05$). Average shoot lengths of *B. rappa* and *L. sativum* seedlings treated with dissolved polymers Polytriticum 2000, BAK402-005, and PVA CP1000 were not significantly decreased as compared with the average shoot lengths for negative controls. Average shoot lengths of *L. sativum* seedlings treated with Polytriticum 2000 were significantly greater as compared with controls ($p<0.05$), suggesting that Polytriticum 2000 decants stimulated plant growth, possibly by providing critical nutritional requirements not present in water or soil used in these experiments.

Conclusions

Our test results indicate that the polymers Polytriticum 2000, BIOPOL D411GN, PVA CP1000, BAK 402-005, and BAK2195/CP1000 (90:10) dissolve in the presence of water. The 24-h decants of the 5 polymers were of relatively low toxicity to the 7 species of aquatic organisms and 2 species of terrestrial plants selected for toxicity testing. A comparison of the study material toxicity values with LC50s and LOECs reported for copper, ammonia, nitrate, sodium chloride, and ethanol suggest that in most cases, these materials are not as toxic as copper, ammonia, or nitrate are to most aquatic organisms, but the test materials are slightly more toxic than sodium chloride or ethanol. Decants of BAK2195/CP1000 (90:10) containing high concentrations of TOC (20 and 40 ppm) inhibited seedling shoot growth of both *B. rappa* and *L. sativum* suggesting that large amounts of BAK2195/CP1000 (90:10) could have an effect on terrestrial plant development and growth. Information gained from these studies will be used for making decisions on which (if any) of the polymers will be suitable for the construction of biodegradable chaff cartridges, pistons, and endcaps. Tensile strength, ease of casting, production waste stream, and overall cost of manufacture are factors that also need to be considered.

GLOSSARY OF TERMS AND ABBREVIATIONS

Note: Common chemical and measurement abbreviations are not included.

d	day
LC50	50% Lethal Concentration
LOEC	Lowest Observable Effective Concentration
NAVAIR	Naval Air Systems
NHRC/TD	Naval Health Research Center Toxicology Detachment
NOEC	No Observable Effective Concentration
TOC	Total Organic Carbon

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INTRODUCTION

Chaff is a radiofrequency (RF) countermeasure released by military aircraft, ships, and vehicles to confuse enemy radar. Chaff consists of aluminum-coated glass fibers ranging in lengths from 0.8 to 0.75 cm and is released in packets of 0.5 to 100 million fibers. Several aircraft types in the U.S arsenal deploy chaff by a cartridge system. Chaff cartridges consist of a hollow tube, an endcap, a piston, pyrotechnic charge or spring, and approximately 0.5 to 1.5 million chaff dipoles (GAO 1998, USAF 1997). The chaff dipoles are released from the cartridge when the spring or pyrotechnic charge pushes the piston down the length of the hollow tube forcing the chaff against the endcap. The endcap then "pops off" and both the chaff and the piston are forced from the chaff cartridge.

At least 500 tons of chaff are released annually during training by U.S. forces (USAF 2001). Discarded styrene dispenser pistons and endcaps account for about 95% of the total mass released to the environment by dispensing chaff (Wilson et al. 2001). Because of their styrene construction, chaff dispenser pistons and endcaps are resistant to dissolution and degradation, accumulate over time, and degrade the quality of the environment (USAF 1997).

Currently, the Navy is experimenting with "biodegradable" chaff dispenser pistons and endcaps constructed with polymers made of natural materials. The Navy plans to field biodegradable chaff dispensers and endcaps by FY 2003. In 1999, U.S. Naval Air Systems (NAVAIR) initiated a program to begin to evaluate the toxicity of several biodegradable

polymers for use in the construction of chaff dispenser pistons and endcaps. Naval Health Research Center Detachment (Toxicology), Wright-Patterson Air Force Base, OH, was asked to carry out these studies on five of the materials under consideration for use in piston and endcap construction. This report summarizes the methods and results of studies designed to characterize the toxicity of these materials in 7 species of aquatic organisms and 2 species of plants.

MATERIALS AND METHODS

1) Test materials

The test polymers were supplied by Naval Aviation Systems (NAVAIR) in a pelletized form with the pellets measuring approximately 5 mm in length and 3 mm in diameter. Polymers used in these tests were Polytriticum 2000, BIOPOL D411GN, BAK 402-005, PVA CP1000, and BAK2195/CP1000 (90:10). Polytriticum 2000 is comprised of wheat starches, wheat protein and biodegradable polyester. BIOPOL D411GN is the random copolymer poly (b-hydroxybutyrate-co-b-hydroxyvalerate) that consists of poly (b-hydroxybutyrate) and poly (b-hydroxyvalerate). BAK 402-005 is a random polyester amide copolymer produced from the monomers hexamethylene diamine [$H_2N(CH_2)_5NH_2$], adipic acid [$HOOC(CH_2)_4COOH$], and butane diol [$HO(CH_2)_4OH$]. PVA CP1000 is a polyvinyl alcohol with the basic structure $[CH_2CHOH]_n$. PVA CP1000 is known to contain internal plasticizers, but the chemistry of these chemicals was not disclosed to the Navy for proprietary reasons. BAK2195/CP1000 (90:10) is a combination of BAK 402-005 and PVA CP1000 in a ratio of 90 parts polyester amide and 10 parts polyvinyl alcohol. Figure 1 shows the chemical structures of the biopolymers and several of their precursors.

2) Measurement of Total Organic Concentrations (TOC)

TOC was measured using a MQ1001 Total Organic Carbon Analyzer described by Qian and Mopper (1996). Aqueous samples were filtered through a 0.45 micron cutoff syringe filter. Approximately 5 ml of sample was transferred to an amber ampule. Ten microliters of 85% phosphoric acid was added to the solution to purge inorganic carbon. The ampules were then flame sealed and stored at -4°C until analysis.

During analysis, sample ampules were opened and then purged with high purity oxygen. The samples were then drawn into a 60 µL sample loop and injected into a combustion column of quartz beads and copper oxide where carbon present in the samples is converted to CO_2. CO_2 levels within the column were measured by non-dispersive IR. Sample CO_2 levels are "calibrated" by comparing sample CO_2 readings with those of standard solutions containing 0-10 mg carbon/L. If necessary, samples were diluted with deionized water if initial readings indicated that TOC levels exceeded the bounds of the calibration curve.

3) Dissolution of Test Materials in Preparation for Toxicity Testing

The water soluble fraction (e.g., the decant) of each material was prepared using a method similar to that used by Anderson et al. (1974) and Fisher et al. (1990). Decants of the test materials were generated by placing 25 grams of polymer in a sealed glass container with one liter of tap water, deionized water, or seawater. The mixture was then stirred continuously for 24 hours. The stirring speed was adjusted to create a vortex that extended approximately one-quarter the distance between the surface of the solution and the bottom of the container. After 24 h of stirring, the containers were allowed to stand for 6 h to allow for settling of particulates.

The supernatant was used as the 100% stock solution. Supernatants and decants were stored at 4°C until needed.

4) Aqueous Dissolution of the Test Materials

Dissolution of the test materials in deionized water or seawater was determined by measuring the average amount of TOC present after immersing the materials for 24 hours, 72 hours, 7 days, 14 days, 30 days, 60 days, or 90 days. Three pellets of each polymer were submerged in 20 ml of deionized water or seawater. Seawater was obtained from the Atlantic Ocean near Ocean City, Maryland, during the early spring of 2001 and filtered with a 0.2 micron filter prior to use. The polymer pellets were placed in water in clear glass vials. All vials were of the same manufacture, thickness, and clarity. Each vial was assigned a study number that was unique for the type of polymer, type of water, and length of incubation time. TOC concentrations were measured for each vial prior to beginning the polymer incubation period. The samples were incubated in a Precision Low Temperature Illuminating Incubator (Model 818) and exposed continuously to fluorescent lighting at a temperature of 45°C. Each sample was removed from the incubator at its designated time point and frozen at -20°C until TOC content could be determined for each vial.

4) Aquatic Toxicity Studies

a. Test Species and Endpoints

The following tests were conducted with freshwater organisms exposed to each test material: 1) 96-h chronic growth test with the green alga *Selenastrum capricornutum*; 2) 48-h acute and 7-d chronic survival and reproduction test with the daphnid *Ceriodaphnia dubia*; 3) 96-h acute and 7-d chronic survival and growth test with the fathead minnow *Pimephales promelas*; and 4) 10-d acute and 28-d chronic survival, growth, and reproductive maturation test with the amphipod *Hyalella azteca*.

The following tests were conducted with saltwater organisms exposed to all materials: 1) 96-h acute and 7-d chronic survival, growth, and fecundity test with the mysid *Americamysis bahia*; 2) 96-h acute and 7-d chronic survival and growth test with the sheepshead minnow *Cyprinodon variegatus*; and 3) 10-d acute and 28-d chronic survival, growth, and reproduction test with the amphipod *Leptocheirus plumulosus*. Aquatic toxicity tests were conducted between February 19, 2001 and August 17, 2001 (see Table 1 for test dates).

b. Test Materials and Stock Solutions

Solutions of each biopolymer were made using the methods described in Section 1 of the Materials and Methods. Stock solutions for all aquatic toxicity testing did not exceed 1% (1 g material:100 g of solution) because of concerns for inducing ion imbalance in the test media (see Goodfellow et al., 2000). The amount of test material that went into solution was a function of the solubility of the material in freshwater, saltwater, or algal assay medium. Stock solutions for algal tests were made by stirring the individual test compounds in 20% Perrier:80% reverse osmosis water on a 1% wt:wt basis. Dilutions of algal test stock solutions were accomplished by adding 20% Perrier:80% reverse osmosis water. Nutrients were added to each dilution as

described by Lewis et al. (1994). The TOC for each stock solution was determined using methods described in Section 2 of the Materials and Methods.

Sediment toxicity tests were conducted by exposing sediment organisms to the lowest no-observed-effect-concentration (NOEC) determined for all aqueous phase organisms on a weight to weight basis (i.e., lowest NOEC among all freshwater and saltwater species for each material). For example, if the aqueous phase NOEC for a compound was 10% material by volume, and it took 10 g of the material per liter to make the 100% water soluble solution, then 1 g of the material was added per 1000 g of sediment. Sediments used in testing were obtained from the Magothy River, MD. Sediment testing was initiated by adding the appropriate amount of biopolymer to each jar containing the test sediment 48 h prior to the addition of organisms. The sediment jars were then hand shaken three times during the next 24 h and then rinsed into glass test beakers with the appropriate overlying water. Overlying water for the freshwater tests consisted of 20% Perrier: 80% reverse osmosis water adjusted to ≈ 2500 µmhos conductivity. Overlying water for the saltwater tests consisted of filtered Wye River estuarine water adjusted to 5‰ with well water. The test beakers were randomized on a temperature controlled test table and allowed to equilibrate for an additional 24 h prior to the addition test organisms and test initiation.

5) Freshwater Toxicity Tests

a. Green Alga

The 96-h chronic toxicity of each test material to the green alga (*S. capricornutum*) was determined by the EPA procedures given in Lewis et al. (1994). Stock algal cultures were reared in 2.5 L Pyrex® culture flasks containing 1 L of sterilized algal assay medium. Cultures were

maintained in a constant temperature incubator under constant cool-white fluorescent lights (~300 foot candles) at a temperature of 25 ± 0.2°C on a shaker table oscillating at 100 ± 10 rpm. Log growth cells were used to start all tests.

Algal test solutions were prepared by diluting algal stock solution as described in Section 4.b. Four replicates were used for each treatment. Test solutions (100 mL total volume) were dispensed into 250 mL Delong flasks and inoculated with S. *capricornutum* cells to achieve a density of ~1 x 10^4 cells/mL. The flasks were placed on a shaker table in an incubator set at the culturing conditions described above. Test solutions were not renewed during the 96-h studies. The endpoint of the test was cell growth. Growth measurements (cell density) were made from all replicates in each treatment at 96 h by counting using a hemocytometer and a light microscope. Test acceptability criteria require cell density in the control flasks to exceed 1 x 10^6 cells/mL at the end of the test and ≤20% variability in the control replicates.

b. Daphnid

The 48-h acute and 7-d chronic toxicity of the five test materials to *C. dubia* were determined by the EPA static renewal method with test solutions renewed daily (Lewis et al. 1994). The daphnid was cultured at 25 ± 1°C in 600 mL glass beakers filled with 400 mL of 20% Perrier:80% reverse osmosis water and fed a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, MO) and green alga (*S. capricornutum*) at a concentration of 120 µg Cerophyl®/mL and ~6.7 x 10^5 *S. capricornutum* cells/mL.

All neonates used in the tests were produced by daphnids in culture that had released at least three broods using the neonate collection procedure outlined in Lewis et al. (1994). The

initial age of the neonates in each test was <24 h old; all hatched in an 8 h window. There were 10 replicates per treatment with each replicate containing one neonate. The tests were conducted in 30 mL polystyrene disposable beakers containing 25 mL of test solution. All tests were conducted for 7 d at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels). All test organisms were fed daily as described above at each 24-h renewal. Routine water quality was determined at the beginning and end of each 24-h renewal. The endpoints of the test were survival and reproduction (i.e., number of neonates). Test acceptability criteria require ≥80% survival and an average of 15 or more young per surviving female in the control treatment. A 48-h LC50 was calculated when possible from the first two days of the chronic exposure.

c. Fathead Minnow

The 96-h acute and 7-d chronic toxicity of the test materials to fathead minnows (*P. promelas*) were determined by the EPA static renewal method (solutions renewed daily) given in Lewis et al. (1994). All larvae used in the tests were <24 h old at the start of the test. The tests were conducted in 600 mL glass beakers containing 300 mL of test solution. Four replicates were used for each treatment with 10 larvae per replicate. The dilution water was a 20% Perrier:80% reverse osmosis water. All test organisms were fed brine shrimp (*Artemia* sp.) nauplii <24 h old twice per day. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. The endpoints of the test were survival and growth. Dry weight at test termination was determined by drying at 100°C for a minimum of 12 h. Test acceptability criteria require ≥80% survival and an average dry weight of ≥0.25 mg in surviving

organisms in the control treatment. A 96-h LC50 was calculated when possible from the first four days of the chronic exposure.

Fathead minnow larvae were obtained from an in-house culture maintained at 25 ± 1°C in non-chlorinated well water. The culture procedures were similar to those recommended by Peltier and Weber (1985). Spawning fish were cultured in 38 L (10 gallon) flow-through glass aquaria supplied with non-chlorinated well water (25 ± 1°C) which was continually filtered and sterilized via ultraviolet light. The spawning adults were fed a diet of TetraMin® Staple Food (Ramfab Aquarium Products Co., Oak Ridge, TN) twice daily. Excess food was removed daily. Eight sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 male:4 females. Replacement spawners were rotated at approximately three-month intervals. Fathead minnow embryos were collected on spawning substrates (10 cm I.D. x 20 cm long PVC pipe sections cut longitudinally in equal portions) and transferred to 19 L aquaria at 25 ± 1°C in unchlorinated well water for hatching. All stages of the fish were reared under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles).

d. Amphipod

The 10-d acute and 28-d chronic toxicity of the test materials to the freshwater amphipod (*H. azteca*) were determined using the static renewal Wye Research and Education Center Standard Operating Procedure (McGee and Fisher, 1998). These methods are consistent with the guidelines outlined in the American Society of Testing and Materials method (ASTM, 1994). The 10-d acute test beakers were renewed twice daily with overlying water while the 28-d chronic test beakers were renewed three times per week. The organisms used for the acute tests were 7 - 14 d old (animals passing through a 710 µm mesh onto a 500 µm mesh) while those used for the chronic tests were approximately one week old (animals passing through a 500 µm mesh onto a 250 µm mesh). The acute test were conducted in 300 mL beakers containing 100 mL sediment and 175 mL overlying water while the chronic tests were

conducted in 1000 mL beakers containing 175 mL sediment and 800 mL overlying water. There were eight replicates containing 10 amphipods each for the acute tests and 5 replicates containing 20 amphipods each for the chronic tests. The overlying water was a 20% Perrier:80% reverse osmosis water adjusted to ≈ 2500 µmhos conductivity. All test organisms were fed a YCT mix (Yeast, Cerophyl®, and Trout Chow). All tests were conducted at 23 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. Overlying ammonia was measured at the beginning and end of each test while porewater ammonia was measured at test initiation.

The endpoints of the test were survival in the acute tests and, survival, growth and reproductive maturation in the chronic tests. Reproductive maturation is a measure of the proportion of females in a treatment that are gravid. All replicate test beakers were sieved through a 500 µm sieve to collect surviving organisms at the end of both the acute and chronic tests. All live animals were counted and, in the chronic test, preserved in 70% ethanol for subsequent estimates of length and sexual maturity. Length was determined by measuring each surviving amphipod from the base of the second antenna to the base of the urosome along the dorsal surface using Sigma Scan Pro 5.0® (SPSS Inc., Chicago, IL). Juvenile amphipods were obtained from Chesapeake Cultures, Hayes, VA. Test acceptability criteria require ≥80% survival in the control treatment in the acute test and ≥80% survival and measurable growth in surviving organisms in the control treatment in the chronic test.

6) Saltwater Toxicity Tests

a. Mysid

The 96-h acute and 7-d chronic toxicity of the five test materials to juvenile mysids (*A. bahia*) were determined by the EPA static renewal method (solutions renewed daily) given in Klemm et al. (1994). All juveniles used in the tests were ≤7 d old at the start of the test. The tests were conducted in 400 mL glass beakers containing 175 mL of test solution. Eight replicates were used for each treatment with 5 mysids per replicate. The salinity of the dilution water was a 20‰. All test organisms were fed brine shrimp (*Artemia* sp.) nauplii <24 h old twice daily. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. The endpoints of the test were survival, growth and fecundity (if ≥50% of females in the control treatments produce eggs). Fecundity is a measure of the proportion of females in a treatment that are carrying eggs. At the termination of the test, all live animals were immediately examined for maturity, males, females with eggs and females without eggs. Dry weight was subsequently determined by drying at 60°C for 24 h. Juvenile mysids were obtained from Chesapeake Cultures, Hayes, VA. All tests were initiated within 24 of receipt of the test organisms. Test acceptability criteria require ≥80% survival and an average dry weight of ≥0.20 mg in surviving organisms in the control treatment. A 96-h LC50 was calculated when possible from the first four days of the chronic exposure.

b. Sheepshead Minnow

The 96-h acute and 7-d chronic toxicity of the five test materials to larval sheepshead minnow (*C. variegatus*) were determined by the EPA static renewal method (solutions renewed daily) given in Klemm et al. (1994). All larvae used in the tests were <24 h old at the start of the test. The tests were conducted in 600 mL glass beakers containing 300 mL of test solution. Four replicates were used for each treatment with 10 larvae per replicate. The salinity of the dilution water was a 20‰. All test

organisms were fed newly hatched brine shrimp (*Artemia* sp.) nauplii <24 h old twice daily. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. The endpoints of the test were survival and growth. Dry weight was determined by drying at 60°C for 24 h. Larval sheepshead minnow were obtained from Aquatic Biosystems, Inc., Fort Collins, CO. Sheepshead minnows from this facility are routinely used in our laboratory for NPDES biomonitoring studies for the Maryland Department of the Environment. All tests were initiated within 24 h of receipt of the test organisms. Test acceptability criteria require ≥80% survival and an average dry weight of ≥0.60 mg in surviving organisms in the control treatment. A 96-h LC50 was calculated when possible from the first four days of the chronic exposure.

c. Amphipod

The 10-d acute and 28-d chronic toxicity of the test materials to the estuarine amphipod (*L. plumulosus*) were determined using the Wye Research and Education Center Standard Operating Procedure (McGee and Fisher, 1998). These method are consistent with the guidelines outlined in the American Society of Testing and Materials method (ASTM, 1994) and the U.S. Environmental Protection Agency methods (U.S. EPA, 1994; 2001). The 10-d acute test beakers were not renewed with overlying water while the 28-d chronic test beakers were renewed three times per week. The organisms used for the acute tests were 2 - 4 mm in length (animals passing through a 710 µm mesh onto a 500 µm mesh) while those used for the chronic tests were approximately 1 - 2 mm (animals passing through a 500 µm mesh onto a 250 µm mesh). Both the acute and chronic tests were conducted in 1000 mL beakers containing 175 mL sediment and 800 mL overlying water. There were five replicates containing 20 amphipods each for both the acute and chronic tests. The overlying water was

filtered Wye River estuarine water adjusted to 5‰ with well water. Acute test organisms were not fed during the test while chronic test organisms were fed three times weekly with TetraMin® dry food ground to ≤250 µm. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. Overlying ammonia was measured at the beginning and end of each test while porewater ammonia was measured at test initiation.

The endpoints of the test were survival in the acute tests and, survival, growth and neonate production in the chronic tests. All replicate test beakers were sieved through a 500 µm sieve to collect surviving adult organisms at the end of both the acute and chronic tests. In addition, the chronic test beakers were sieved through a 250 µm sieve to collect neonates produced during the tests. The neonates were preserved in 70% ethanol with rose bengal for subsequent counting. Dry weight was determined by drying at 100°C for at least 24 h. Test acceptability criteria require ≥90% survival in the control treatment in the acute test and ≥80% survival and measurable growth and reproduction in surviving organisms in the control treatment in the chronic test.

Juvenile amphipods were obtained from an in-house culture maintained at 25 ± 1°C in overlying estuarine water adjusted to 5‰ with well water under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Culture procedures are outlined in McGee and Fisher (1998) and are consistent with procedures presented in the U.S. Environmental Protection Agency guuidelines (U.S. EPA, 2001). Individual cultures were maintained in plastic wash basins (34 cm x 28 cm x 13 cm deep) containing a fine-grained, clayey silt sediment from the Magothy River, MD. The cultures were fed 0.4 g of finely milled TetraMin® dry food three times per week and the overlying water was replaced three

times per week. The cultures were thinned and the sediment replaced every 6 to 8 weeks. Test organisms were obtained from the cultures by passing the sediment through the appropriate sieves as described above.

7) Shoot Emergence and Length

Tests for shoot emergence and length were conducted in *Brassica rappa* and *Lepidium sativum* using methods outlined by ASTM (1999) with modification. Seeds of *B. rappa* or *L. sativum* were planted in pots (volume: 216 cm^3) containing 200 g of commercial potting soil, at a depth of 1.0 cm. Ten seeds were planted in each pot. For each test, four pots were designated negative controls and were given filtered tap water (75mL) once every 24 hours for 5 days. Four pots were designated positive controls and were given 75 mL of boric acid solutions in tap water at concentrations of 40, 80, 160, or 320 ppm, once every 24 hours for 5 days. Test pots were watered each day with 75 mL of test solution at concentrations of 50 or 100% mixed with filtered tap water, once every 24 hours for 5 days. All test materials were assayed in duplicate in each test. Each material was assayed twice.

For each test, all pots were placed in a low temperature illuminated incubator (Precision, Inc.) with constant lighting and an average internal temperature of 20°C. All pots were covered with plastic wrap until emerging seedlings were observed. All pots were removed from the incubator at the same time for watering every 24 hours. Temperature, humidity levels, and the number of emergent seedlings were recorded once every 24 hours. Figure 2 shows a typical experiment being carried out in the low temperature illuminated incubator.

On day 6, the number of emergent seedlings was recorded for each pot. Shoot length was measured and recorded for each seedling. Seedling length was determined by measuring the distance between the base of the first cotyledon and the transition point between hypocotyl and root. Percent emergence and average shoot length was determined by dividing each parameter by the number of emergent plants in the particular pot. Soil pH was determined for all pots on Day 6 using methods recommended by ASTM (1999).

8) Data Analyses

a. Aqueous Dissolution Results and Shoot Emergence and Length

Statistical analysis of the aqueous dissolution results and plant toxicity data was carried out using StatView statistical analysis software (SAS 1999). For the biopolymer dissolution studies, percent TOC levels were compared between treatment groups using ANOVA. Percent emergence and seedling lengths were compared between treatment groups using ANOVA. If a significant difference was found for the between groups comparison ($p<0.05$), further analyses were carried out to determine which experimental groups differed significantly from controls ($p<0.05$) using Fisher's Exact Test and the Bonferroni/Dunn post-hoc multiple comparison tests.

b. Aquatic toxicity studies

The effect concentration for each compound and species is presented as the percentage of the water soluble fraction of each stock solution as recommended by the USEPA (Lewis et al., 1994). LC50s (and their 95% confidence limits) were determined by the Trimmed Spearman-Karber method in

the cases where toxicity occurred (U.S. EPA, 1993). The mortality data used for calculating the LC50s were taken from data generated during the early portions of the respective chronic tests. The no-observed-effect concentration (NOEC) and lowest-observed-effect concentrations (LOEC) for all of the chronic tests and for the acute and chronic sediment tests were determined by the methods outlined in Lewis et al. (1994). Statistical tests were performed using TOXSTAT 3.5 (WEST and Gulley, 1994) at a minimum probability level of 0.05. In summary, all proportion survival data were arc-sine square root transformed prior to analysis using a procedure that adjusts for proportion survival of 1.0 (100% survival) and 0 (100% mortality). The proportion data generated for the mysid fecundity and freshwater amphipod reproductive maturity endpoints were arc-sine square root transformed without this adjustment because the number of females in each treatment could differ. In addition, the adult daphnid survival data were analyzed by Fisher's Exact Test without transformation. All other data were not transformed prior to analysis.

All data were analyzed for normality (Shapiro-Wilk's Test) and homogeneity of variances (Bartlett's Test) prior to testing differences between treatments. If the data passed the normality and homogeneity tests they were analyzed by a simple t-test or analysis of variance (ANOVA) followed by a Dunnett's Test if there were an equal number of replicates in each treatment or a t-test with Bonferroni Adjustment if replicate sizes were unequal. If the normality and/or the homogeneity of variances tests failed, the data were analyzed using the non parametric Steel's Many One Rank Test for equal replicate sizes or the Wilcoxon Rank Sum Test for unequal replicate sizes. Table 2 outlines all of the statistical tests conducted on all of the endpoints tested for each compound.

RESULTS

1) Aqueous Dissolution of Test Materials

Comparison of TOC levels showed that significantly greater amounts of organic carbon were present in deionized water and seawater after incubation for greater than 24 hours for polymers Polytriticum 2000, BAK402-005, PVA CP1000, BAK 2195/CP1000 (90:10) as compared with the amounts of organic carbon present at the beginning of the dissolution experiment (Tables 3 and 4). The amounts of TOC measured for vials containing BIOPOL D411GN did not increase with increasing incubation time indicating that substantial amounts of this material did not dissolve when placed in either deionized water or seawater for up to 90 days.

2) Aquatic Toxicity Studies

Tables 5 – 9 summarize the aquatic toxicity testing results. BIOPOL D411GN was found to be toxic to fathead minnow, sheepshead minnow, the mysid *A. bahia*, the daphnid *C. dubia*, and the algae *S. capricornutum* at concentrations ranging from 1.24 – 9.90 mg/L TOC representative of dissolved BIOPOL D411GN (Table 5). Polytriticum 2000 was found to be toxic to fathead minnow, sheepshead minnow, the mysid *A. bahia*, and the daphnid *C. dubia* at concentrations ranging from 2.74 – 9.20 mg/L TOC representative of dissolved Polytritcum 2000 (Table 6). BAK 2195/CP1000 (90:10) was not toxic to the freshwater organisms fathead minnow, algae *S. capricornutum*, the daphnid *C. dubia*, and the amphipod *H. azteca* at up to 18.00 mg/L of TOC representative of dissolved BAK 2195/CP1000 (90:10)

(Table 7). BAK 2195/CP1000 (90:10) was not toxic to the saltwater aquatic organism's sheepshead minnow, the mysid *A. bahia*, and the amphipod *L. plumulosus* at up to 17.80 mg/L of TOC representative of dissolved BAK 2195/CP1000 (90:10) (Table 7). BAK402-005 was toxic to the mysid *A. bahia* at 10.70 mg/L of TOC representative of dissolved BAK 402-005 and the algae *S. capricornutum* at a concentration of 731.30 mg/L of TOC representative of dissolved BAK 402-005 (Table 8). BAK 402-005 was not toxic to the freshwater organisms the fathead minnow and the daphnid *C. dubia* at concentrations of up to 731.30 mg/L, and was not toxic to the freshwater amphipod *H. azteca* at up to concentrations of 409.52 mg/L. BAK 402-005 was not toxic to the sheepshead minnow at average concentrations of up to 19.10 mg/L. PVA CP1000 was toxic to two organisms, the mysid *A. bahia* and the daphnid *C. dubia*, at average concentrations ranging from 3.90 – 14.31 mg/L and 9.87 – 172.64 mg/L of TOC representative of dissolved PVA CP1000, respectively (Table 9). PVA CP1000 was toxic to the algae *S. capricornutum* at an average concentration of up to 308.30 mg/L TOC. PVA CP1000 was not toxic to fathead or sheepshead minnow at average concentrations of up to 308.30 or 39.00 mg/L TOC, respectively, or the amphipods *H. azteca* or *L. plumulosus* at average concentrations of up to 5.55 or 0.70 mg/L TOC, respectively (Table 9).

3) Shoot Emergence and Root Elongation

Results of percent emergence test results for *B. rappa* are summarized in Table 10. Treatment of *B. rappa* seeds with increasing concentrations of boric acid in water resulted in significantly ($p<0.05$) smaller number of plants emerging by Day 6 of the study (Figure 3). Percent emergence of *B. rappa*

was significantly lower ($p<0.05$) for seeds treated with Polytriticum 2000 at a water TOC concentration of 11.59 ppm. Percent emergence of *B. rappa* was also significantly lower ($p<0.05$) for seeds treated with BIOPOL D411GN at a water TOC concentration of 3.8 ppm. In both cases, however, there was no evidence of a dose-response since percent seed emergence was not significantly different from negative controls with increasing amounts of chemical applied to the soil as observed for seeds treated with increasing amounts of boric acid.

The average shoot length of *B. rappa* negative controls and of plants treated with boric acid and the various biopolymers are summarized in Table 11. Average shoot lengths of seeds treated with boric acid were consistently and significantly ($p<0.05$) lower than the average lengths for negative control plants. Increasing amounts of boric acid applied to the soil resulted in a concentration-dependent decrease in average shoot length (Figure 4). The average shoot length for *B. rappa* was significantly ($p<0.05$) lower for seeds treated with BAK2195/CP1000 (90:10) decant as compared with negative controls and was concentration dependent (Figure 4). Average shoot length for seeds treated with BIOPOL D411GN at a TOC concentration of 5.0 ppm were significantly ($p<0.05$) decreased as compared with negative controls according to Fischer's Exact Test, but not the more stringent Bonferroni/Dunn test for multiple comparisons. Since greater amounts of BIOPOL D411GN decant were not tested, it cannot be determined from this data whether the observed decrease was treatment-related or a random finding.

A statistically significant difference ($p<0.0001$) in emergence was found between experimental groups for *L. sativum* (Table 12). Application of boric acid to watercress seeds at concentration levels of 160 and 320 ppm resulted in significantly fewer seeds emerging as compared with negative controls.

Percent emergence of *L. sativum* seeds treated with 160 and 320 ppm boric acid was 5.8 and 2.0, respectively, as compared with a percent emergence of 7.6 for negative controls (Table 12). Application of boric acid at 40 and 80 ppm resulted in a significantly higher percent emergence of *L. sativum* as compared with negative controls. The percent emergence of *L. sativum* treated with 80 and 40 ppm boric acid was 8.6 and 9.4 as compared with 7.6 for negative controls.

Percent emergence of *L. sativum* seeds was not affected in a manner indicative of toxicity. Figure 5 shows the effect of the application of increasing amounts of biopolymer decant and boric acid on percent emergence of *L. sativum*. When compared with the average percent emergence of the negative controls, in most cases the percent emergence of seeds treated with biopolymer decants are higher. Percent emergence was significantly greater for *L. sativum* treated with 50:50 mixtures of tap water and either Polytriticum 2000 or BIOPOL D411GN.

Average shoot lengths of *L. sativum* between the various treatment groups was found to be significantly different (Table 13). Average shoot length of emergent *L. sativum* treated with 80, 160, or 320 ppm boric acid was 0.4, 0.6, and 1.4 centimeters as compared with an average of 2.4 centimeters for negative controls ($p<0.05$). Average shoot lengths of *L. sativum* treated with 50 or 100% decants of BAK2195/CP1000 (90:10) were significantly decreased as determined by the Fisher's Exact Test ($p<0.05$). A number of polymer treatments were associated with significantly higher average shoot lengths as compared with negative controls (Figure 6). Average shoot lengths of *L. sativum* treated with decants of Polytritcum 2000 were significantly elevated as compared with controls as determined by the Fisher's Exact Test ($p<0.05$). Average shoot length of *L. sativum* treated with 50 and 75% Polytritcum 2000 decants were significantly higher ($p<0.002$) than those of the negative controls when compared

using the Bonferroni/Dunn test for multiple comparisons. Average shoot lengths for of *L. sativum* treated with polyvinyl alcohol followed a bimodal dose-effect with higher average shoot lengths occurring for *L. sativum* treated with 25, 50, and 75% decants and a significantly decreased average shoot length for seeds treated with the 100% polyvinyl alcohol (BAK 402-005) decant.

DISCUSSION

1) Aqueous Dissolution of Test Materials

TOC data suggest that Polytriticum 2000, BAK402-005, PVA CP1000, and BAK 2195/CP1000 (90:10) dissolve in deionized water or seawater when placed in these solvents for up to 90 days under static conditions. In many instances, however, there was no consistent evidence of a linear relationship between increasing amounts of TOC with increasingly longer incubation times. The large confidence intervals for average TOC levels for many of the time points suggests that either there was significant instrument or operator error associated with determining TOC levels or that dissolution levels varied widely between samples of the same chemical with the same incubation time periods.

TOC data suggests that very little BIOPOL D411GN dissolves when placed in deionized water or seawater for up to 90 days. This finding was expected since BIOPOL D411GN is described by its manufacturer as a complex copolymer. Although we found evidence that BIOPOL D411GN did not dissolve appreciably, it is possible that greater amounts of these materials could dissolve if placed in H_2O for longer than 90 days.

2) Aquatic Toxicity Studies

Two aquatic toxicity tests did not meet test acceptability criteria. The *H. azteca* 28-d chronic test did not meet the test acceptability criteria of ≥ 80% survival in controls. Survival of *H. azteca* was 56%, however, *H. azteca* length and reproduction parameters among surviving organisms was within the

historical range expected for untreated control organisms. We speculate that shaking the sediment during this test may have produced a flocculent like sediment unacceptable to this species during long exposures. The sediment never consolidated over the course of the 28-d study. A light fluffy layer approximately 2 to 3 cm in depth covering a denser layer on the bottom of the test beakers was present throughout the test. The same sediment preparation method was used in both the acute and chronic saltwater tests with *L. plumulosus* and the freshwater acute test with *H. azteca*. All of the sediment tests with the exception of the 28-d *H. azteca* met test acceptability criteria. *L. plumulosus* is a burrowing amphipod and it did not seem to have problems working through the sediment and establishing burrows deeper in the layers. Since *H. azteca* is epibenthic it spends most of its time on the surface of the sediment grazing for food. The texture of the sediment could have affected its ability to maintain contact with the surface over the longer test period of the chronic test. Concurrent with the chaff material test, our laboratory also conducted an ambient toxicity test for another sponsor on unshaken sediments. The control sediment was the same as that used in the endcap test, without shaking. The *H. azteca* in this unmixed control sediment did well, with 89% survival at 28 days. It appears that in an effort to distribute the endcap materials evenly throughout the sediment an environment was created which was unacceptable to the amphipod for long term survival. Since all of the other sediment toxicity tests showed no toxicity, even the acute *H. azteca* test, and there was no difference in survival, length and reproduction in surviving amphipods in the 28-d test, it appears that the endcap materials are not toxic in sediment exposures.

There were also some problems with toxicity tests of BAK 2195/CP1000 (90:10) in mysid. Only 30.9% of the control mysid females produced eggs in tests conducted with BAK 2195/CP1000 (90:10), therefore, fecundity could not be used as an endpoint for determining the toxicity of BAK 402/CP1000

decant fractions. The test criteria require a minimum fecundity of ≥50% among female controls. According to USEPA, failure to meet the fecundity requirement does not invalidate toxicity data gained from this test for other endpoints (Klemm et al., 1994). All other acceptability criteria for this test were met; therefore, the 96-h LC50s calculated from these tests were considered valid test results.

It is critical to compare the toxicity values of the tested chemicals with toxicity values of chemicals commonly found in the aquatic environment to be better able to gauge the relative danger of the tested materials to aquatic organisms. Table 14 lists the toxicity values of several chemicals commonly found in aquatic environments. LC50 and other available data were gathered by accessing USEPA's ECOTOX Database System which is a compilation of aquatic toxicity values for species commonly used in aquatic toxicity testing. Copper is a common aquatic contaminate and toxic to most aquatic species at microgram levels (Table 14). Sodium chloride and ethanol are found naturally in aquatic environments and LC50s for these chemicals range between 1 to 18 grams in fathead minnow and *Ceriodaphnia*. Ammonia and nitrate are common contaminates in aquatic ecosystems and have LC50s that fall between the values for copper and sodium chloride/ethanol for most aquatic organisms.

Our comparison suggests that the 24-h decants of the materials tested in this study are not very toxic in comparison to chemicals commonly found in aquatic environments. In our study, the five polymers tested were toxic to 7 species of aquatic organisms at an average concentration range of 1.24 – 731.30 mg/L TOC. BAK2195/CP1000 was not toxic to any of the organisms under the given test conditions. A comparison of the study material toxicity values with LC50s and LOECs reported for copper, ammonia, nitrate, sodium chloride, and ethanol suggest that in most cases, these materials are not as toxic as

copper, ammonia, or nitrate, but are slightly more toxic than sodium chloride or ethanol are to most aquatic organisms.

3) Shoot Emergence and Root Elongation

Both *B. rappa* or *L. sativum* were sensitive to boric acid exposure as indicated by the decreasing percentage of seed emergence and shoot lengths with increasing concentration of these chemicals in water. This finding demonstrates that both *B. rappa* or *L. sativum* are sensitive to the toxicity of boric acid under our laboratory conditions and show that seed emergence and shoot length are valid endpoints with which to measure the effects of our test materials on the development and growth of these plants. High soil concentrations of boron are known to be toxic to a number of plants (Woods 1994). The mechanism by which excess boron causes toxicity in plants is largely unknown, but it is speculated that boron may interfere with various aspects of plant metabolism including photosynthesis (Blevins and Lukaszewski 1994). Boron is an essential micronutrient for higher plants in low concentrations (Blevins and Lukaszewski 1994) which may explain why average seed emergence of *L. sativum* was higher for seeds treated with 40 or 80 ppm boric acid in tap water as compared with negative controls given tap water only.

There are very few reports in the peer-reviewed scientific literature on the effects of polymer exposure to plants. Grazia et al. (2000) showed that *Allium cepa* bulbs developed significantly greater numbers of root tip cytogenic aberrations when grown in mineral water previously contained in polyethylene terephthalate bottles for 8 weeks as compared with bulbs grown in mineral water contained

in glass containers over the same 8 weeks. Several polyester hydrolysis products including succinic acid, adipic acid, mandelic acid, and others were found to inhibit germination of radish seeds (Kim et al. 2001). Direct contact with polyurethane foam was found to be toxic to young lettuce, red beet, and potato plant seedlings in solution culture (Wheeler et al. 1985).

There is some evidence from our findings that treatment of *B. rappa* or *L. sativum* seeds with biopolymer decants may have stimulated seed emergence and shoot growth. Percent seed emergence and average shoot length of *L. sativum* treated with polyvinyl alcohol decant were, on average, at least 20% higher as compared with controls. Percent emergence of *B. rappa* was elevated for several biopolymer decants (Figure 3), but not significantly. Average shoot length of *B. rappa* treated with BAK2195/CP1000 (90:10) was greater than 20% than the average shoot length of negative controls (Figure 4). It is possible that elements present in the biopolymer decant stimulated growth by meeting critical nutritional requirements that were not being met in the negative controls or other biopolymer treatment groups. The shoot lengths of *L. sativum* seedlings treated with Polytriticum 2000 were on average 70% longer than those of negative control seedlings (Table 13).

CONCLUSIONS

Our testing results indicate that the polymers Polytriticum 2000, BIOPOL D411GN, PVA CP1000, BAK 402-005, and BAK2195/CP1000 (90:10) dissolve in water over time under static conditions and that the concentrations of total organic carbon (TOC) that are found in the decant of the material after 24 hours in water are virtually nontoxic to 7 species of aquatic organisms and 2 species of terrestrial plants. The results of our testing indicate that the polymers Polytriticum 2000, BIOPOL D411GN, PVA CP1000, BAK 402-005, and BAK2195/CP1000 (90:10) dissolve in the presence of water. The 24-h decants of the 5 polymers were of relatively low toxicity to the 7 species of aquatic organisms and 2 species of terrestrial plants selected for toxicity testing. A comparison of the study material toxicity values with LC50s and LOECs reported for copper, ammonia, nitrate, sodium chloride, and ethanol suggest that in most cases, these materials are not as toxic as copper, ammonia, or nitrate are to most aquatic organisms, but are slightly more toxic than sodium chloride or ethanol. Decants of BAK2195/CP1000 (90:10) containing high concentrations of TOC (20 and 40 ppm) inhibited seedling shoot growth of both *B. rappa* and *L. sativum* suggesting that large amounts of BAK2195/CP1000 (90:10) could have an effect on terrestrial plant development and growth. Although a relatively diverse range of aquatic organisms and two species of terrestrial plants were used in these studies, our characterization of the ecotoxicity of the polymers evaluated in these studies may not be applicable to all species or environmental situations. Information gained from these studies will be used for making decisions on which (if any) of the polymers will be suitable for the construction of biodegradable chaff cartridges, pistons, and endcaps. Tensile strength, ease of casting, production waste stream, and overall cost of manufacture are factors that also need to be considered.

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Table 1: Dates of aquatic toxicity tests by polymer conducted at the Wye Research Institute (University of Maryland)

Compound	Species	Test Date
BIOPOL D411GN	Fathead minnow	3/23 - 3/3-/01
	Ceriodaphnia	5/3 -5/1-/01
	Green Algae	5/1 - 5/5/01
	Sheepshead minnow	3/23 - 3/30/01
	Mysid	3/23 - 3/30/01
Polytriticum 2000	Fathead minnow	3/23 - 3/30/01
	Ceriodaphnia	5/3 - 5/10/01
	Green Algae	2/19 - 2/23/01
	Sheepshead minnow	3/23 - 3/30/01
	Mysid	3/23 - 3/30/01
BAK 2195/CP1000 (90:10)	Fathead minnow	4/8 - 4/15/01
	Ceriodaphnia	4/8 - 4/15/01
	Green Algae	5/1 - 5/5/01
	Sheepshead minnow	5/18 - 5/25/01
	Mysid	5/18 - 5/25/01
BAK 402 – 005	Fathead minnow	6/1 - 6/8/01
	Ceriodaphnia	6/2 - 6/9/01
	Green Algae	5/26 - 5/30/01
	Sheepshead minnow	6/1 - 6/8/01
	Mysid	6/1 - 6/8/01
PVA CP1000	Fathead minnow	6/1 - 6/8/01
	Ceriodaphnia - Acute	6/2 - 6/4/01
	Ceriodaphnia - Chronic	6/23 - 6/30/01
	Green Algae	5/26 - 5/30/01
	Sheepshead minnow	6/1 - 6/8/01
	Mysid - Acute	6/2 - 6/5/01
	Mysid - Chronic	6/22 - 6/29/01
Sediment Freshwater Acute Tests - All	*Hyalella azteca*	7/20 - 7/30/01
Sediment Freshwater Chronic Tests - All	*Hyalella azteca*	7/20 - 8/17/01
Sediment Saltwater Acute Tests - All	*Leptocheirus plumulosus*	7/20 - 7/30/01
Sediment Saltwater Chronic Tests - All	*Leptocheirus plumulosus*	7/20 - 8/17/01

Table 2: Statistical analyses used in each chaff endcap test; $\propto = 0.05$; t-test with Bonferroni or Wilcoxon used if unequal replicates

Compound	Species	Endpoint	Normality Shapiro-Wilk's	Homogeneity Bartletts	Fisher's Exact	Parametric ANOVA			Non parametric	
			Chi-Square for Mysid/ Ceriodaph			Dunnett's	t-test	t-test Bonferroni Adjustment	Steel's Many One Rank	Wilcoxon Rank Sum
BIOPOL D411GN	Fathead minnow	Survival	Pass	Fail					_	
		Growth	Pass	Pass		_				
	Ceriodaphnid	Survival	Pass	Pass	_					
		Reproduction	Pass	Pass		_				
	Algae	Growth	Pass	Pass		_				
	Sheepshead minnow	Survival	Pass	Fail					_	
		Growth	Pass	Pass		_				
	Mysid	Survival	Fail	Pass					_	
		Growth	Pass	Pass		_				
		Reproduction	Fail	Pass						_
Polytriticum 2000	Fathead minnow	Survival	Fail	Fail						
		Growth	Pass	Pass		_				
	Ceriodaphnid	Survival	Pass	Pass	_					
		Reproduction	Pass	Pass		_				
	Algae	Growth	Pass	Pass			_			
	Sheepshead minnow	Survival	Fail	Fail					_	
		Growth	Pass	Pass		_				
	Mysid	Survival	Fail	Pass					_	
		Growth	Pass	Fail					_	
		Reproduction	Pass	Pass		_				

Table 2: (Continued). Statistical analyses used in each chaff endcap test; $\propto = 0.05$; t-test with Bonferroni or Wilcoxon used if unequal replicates

Compound	Species	Endpoint	Normality Shapiro-Wilk's	Homogeneity Bartletts	Fisher's Exact	Parametric ANOVA			Non parametric	
			Chi-Square for Mysid/ Ceriodaph			Dunnett's	t-test	t-test Bonferroni Adjustment	Steel's Many One Rank	Wilcoxon Rank Sum
BAK 2195/ CP1000 (90:10)	Fathead minnow	Survival	Pass	Pass		_				
		Growth	Pass	Pass		_				
	Ceriodaphnid	Survival	Pass	Pass	_					
		Reproduction	Pass	Pass		_				
	Algae	Growth	Pass	Pass		_				
	Sheepshead minnow	Survival	Fail	Fail					_	
		Growth	Pass	Pass		_				
	Mysid	Survival	Fail	Pass					_	
		Growth	Pass	Pass		_				
		Reproduction	Fail	Pass						_
BAK 402-005	Fathead minnow	Survival	Pass	Pass						
		Growth	Pass	Pass		_				
	Ceriodaphnid	Survival	Pass	Pass	_					
		Reproduction	Pass	Pass		_				
	Algae	Growth	Pass	Pass		_				
	Sheepshead minnow	Survival	Pass	Fail					_	
		Growth	Pass	Pass		_				
	Mysid	Survival	Fail	Fail					_	
		Growth	Pass	Pass		_				
		Reproduction	Pass	Pass				_		

Table 2: (Continued). Statistical analyses used in each chaff endcap test; $\propto = 0.05$; t-test with Bonferroni or Wilcoxon used if unequal replicates

Compound	Species	Endpoint	Normality Shapiro-Wilk's	Homogeneity Bartletts	Fisher's Exact	Parametric ANOVA			Non parametric	
			Chi-Square for Mysid/ Ceriodaph			Dunnett's	t-test	t-test Bonferroni Adjustment	Steel's Many One Rank	Wilcoxon Rank Sum
PVA CP1000	Fathead minnow	Survival	Pass	Pass		_				
		Growth	Pass	Pass			_			
	Ceriodaphnid	Survival	Pass	Fail	_					
		Reproduction	Pass	Fail					_	
	Algae	Growth	Pass	Pass		_				
	Sheepshead minnow	Survival	Pass	Fail					_	
		Growth	Pass	Pass		_				
	Mysid	Survival	Fail	Fail					_	
		Growth	Pass	Pass		_				
		Reproduction	Fail	Fail						_
Sediment Tests	*Hyalella* - Acute	Survival	Fail	Pass						
	Hyalella - Chronic	Survival	Pass	Pass		_				
		Growth	Pass	Pass		_				
		Reproduction	Pass	Fail					_	
	Leptocheirus - Acute	Survival	Fail	Pass					_	
	Leptocheirus - Chronic	Survival	Pass	Pass		_				
		Growth	Pass	Pass		_				
		Reproduction	Pass	Pass		_				

Table 3: TOC concentrations for biodegradable polymers following incubation in deionized water for up to 90 days			
Incubation Period	N	TOC concentration (mg/L)	95% CI
BIOPOL D411GN			
Start	3	9.5	8.75 -10.25
24	3	4.8*,**	4.30 - 5.30
72	2	11.1*,**	4.81 – 17.89
168	3	5.4*,**	4.65 - 6.15
336	2	5.7*,**	4.80 - 6.60
720	2	7.1*,**	2.61 - 11.50
1440	-	-	-
2160	3	5.3*,**	4.40 - 6.20
Polytriticum 2000			
Start	2	7.0	3.41 – 10.59
24	3	20.5*,**	19.01 – 21.99
72	3	22.6*,**	21.61 – 23.59
168	3	36.8*,**	32.08 – 41.52
336	3	19.2*,**	17.71 – 20.69
720	3	5.3	4.80 – 5.80
1440	3	13.6*	3.66 – 23.54
2160	3	31.9*,**	30.91 – 32.89
PVA CP1000			
Start	3	6.2	5.45 – 6.95
24	3	13.6	13.10 - 14.10
72	3	21.9	20.16 – 23.64
168	3	62.6*,**	58.63 – 66.57
336	3	35.9*	34.41 – 37.39
720	3	62.3*,**	58.33 – 66.27
1440	3	56.4*,**	52.67 – 60.13
2160	3	171.2*,**	96.42 – 245.98
BAK 402-005			
Start	2	6.2	3.50 – 8.90
24	3	16.8*	15.31 – 18.29
72	3	21.5*	11.62 – 31.38
168	3	25.4*	24.41 – 26.39
336	3	22.6*	21.36 – 23.84
720	3	47.6*,**	43.38 – 51.82
1440	3	57.1*,**	36.73 – 77.47
2160	3	32.1*,**	19.43 – 44.77
BAK 2195/CP1000 (90:10)			
Start	3	10.3	8.56 – 12.04
24	3	24.7*	23.56 – 25.94
72	3	31.0*	25.53 – 36.47
168	3	31.9*	27.93 – 35.87
336	3	32.8*	28.08 – 37.52
720	3	71.2*,**	62.75 – 79.65
1440	3	55.9*,**	50.68 – 61.12
2160	3	69.5*,**	25.53 – 113.47
* Difference as compared with TOC concentration at time zero is statistically significant by Fisher's Exact Test, $p<0.05$			
** Difference as compared with TOC concentration at time zero is statistically significant by Bonferroni/Dunn Test, $p<0.001$			

Table 4: TOC concentrations for biodegradable polymers following incubation in sea water for up to 90 days

Incubation Period	N	TOC concentration (mg/L)	95% CI
BIOPOL D411GN			
Start	3	21.3	18.07 – 24.53
24	2	7.2[*,**]	2.71 – 11.69
72	2	6.6[*,**]	3.90 – 9.30
168	3	4.6[*,**]	4.35 – 4.85
336	2	6.6[*,**]	4.80 – 8.40
720	3	8.2[*,**]	6.71 – 9.69
1440	3	10.1[*,**]	7.62 – 12.58
2160	3	5.3[*,**]	5.05 – 5.55
Polytriticum 2000			
Start	2	11.4	3.31 – 19.49
24	3	20.5[*]	17.62 – 23.58
72	3	22.1[*]	21.61 – 23.59
168	3	17.5	21.11 – 23.09
336	3	9.4	8.65 – 10.15
720	3	15.2	14.21 – 16.19
1440	3	109.5[*,**]	87.89 – 131.11
2160	3	32.0[*,**]	29.76 – 34.24
PVA CP1000			
Start	2	11.0	0.00 – 22.68
24	3	12.8	11.56 – 14.04
72	3	17.3	14.57 – 20.03
168	3	27.5[*]	22.53 – 32.47
336	3	33.7[*]	31.22 – 36.18
720	3	60.0[*,**]	49.57 – 70.43
1440	3	30.9[*]	27.42 – 34.38
2160	3	65.4[*,**]	20.19 – 110.61
BAK 402-005			
Start	3	6.6	4.86 - 8.34
24	3	11.4[*,**]	9.66 – 13.44
72	3	22.4[*,**]	18.18 – 26.62
168	3	43.3[*,**]	37.59 – 49.01
336	3	36.7[*,**]	32.48 – 40.92
720	3	39.6[*,**]	35.63 – 43.57
1440	-	-	-
2160	3	41.5[*,**]	37.77 – 45.23
BAK 2195/CP1000 (90:10)			
Start	3	8.2	7.70 – 8.70
24	3	19.4[*,**]	16.92 – 21.88
72	3	36.6[*,**]	33.12 – 40.08
168	3	30.0[*,**]	25.78 – 34.22
336	3	69.6[*,**]	58.92 – 80.28
720	2	83.7[*,**]	2.84 – 164.56
1440	3	65.4[*,**]	63.41 – 67.39
2160	3	72.1[*,**]	50.49 – 93.71

[*] Difference as compared with TOC concentration at time zero is statistically significant by Fisher's Exact Test, $p<0.05$

[++] Difference as compared with TOC concentration at time zero is statistically significant by Bonferroni/Dunn Test, $p<$

Table 5: Test results for BIOPOL D411GN water column and sediment toxicity tests

Species - Test – Endpoint			BIOPOL D411GN
Fathead minnow (*Pimephales promelas*)			
Acute (96-h LC50)			7.15 mg/L (72.2%)[1]
Chronic (Survival)		NOEC	5.54 mg/L (56.0%)
		LOEC	9.90 mg/L (100.0%)
Chronic (Growth)	NOEC		3.17 mg/L (32.0%)
		LOEC	5.54 mg/L (56.0%)
Sheepshead minnow (*Cyprinodon variegatus*)			
Acute (96-h LC50)			9.21 mg/L (74.3%)
Chronic (Survival)		NOEC	6.94 mg/L (56.0%)
		LOEC	12.4 mg/L (100.0%)
Chronic (Growth)	NOEC		3.97 mg/L (32.0%)
		LOEC	6.94 mg/L (56.0%)
Mysid (*Americamysis bahia*)			
Acute (96-h LC50)			6.67 mg/L (53.8%)
Chronic (Survival)		NOEC	3.97 mg/L (32.0%)
		LOEC	6.94 mg/L (56.0%)
Chronic (Growth)	NOEC		0.69 mg/L (5.6%)
		LOEC	1.24 mg/L (10.0%)
Chronic (Fecundity)		NOEC	1.24 mg/L (10.0%)
		LOEC	2.23 mg/L (18.0%)
Daphnid (*Ceriodaphnia dubia*)			
Acute (48-h LC50)			Not acutely toxic at 9.90 mg/L (100%)
Chronic (Survival)		NOEC	Not chronically toxic at 9.90 mg/L (100%)
Chronic (Reproduction)		NOEC	5.54 mg/L (56%)
		LOEC	9.90 mg/L (100%)
Algae (*Selenastrum capricornutum*)			
		NOEC	1.78 mg/L (18%)
		LOEC	3.17 mg/L (32%)
Amphipod *Hyalella azteca* 10-d (survival)			Not acutely toxic at 0.55 mg/L (5.6%)[2]
Amphipod *Hyalella azteca* 28-d (survival, growth, reproduction)			Not chronically toxic at 0.55 mg/L (5.6%)
Amphipod *Leptocheirus plumulosus* 10-d (survival)			Not acutely toxic at 0.69 mg/L (5.6%)
Amphipod *Leptocheirus plumulosus* 28-d (survival, growth, reproduction)			Not chronically toxic at 0.69 mg/L (5.6%)

[1]Water column toxicity expressed as % stock solution by volume based on total organic carbon
[2]Sediment toxicity expressed as percent compound in sediment by weight

Table 6: Test results for Polytriticum 2000 water column and sediment toxicity tests

Species - Test – Endpoint			Polytriticum 2000
Fathead minnow (*Pimephales promelas*)			
Acute (96-h LC50)			Not acutely toxic at 9.20 mg/L (100%)[1]
Chronic (Survival)		NOEC	5.15 mg/L (56%)
		LOEC	9.20 mg/L (100%)
Chronic (Growth)	NOEC		2.94 mg/L (32%)
		LOEC	5.15 mg/L (56%)
Sheepshead minnow (*Cyprinodon variegatus*)			
Acute (96-h LC50)			Not acutely toxic at 4.90 mg/L (100%)
Chronic (Survival)		NOEC	2.74 mg/L (56%)
		LOEC	4.90 mg/L (100%)
Chronic (Growth)	NOEC		1.57 mg/L (32%)
		LOEC	2.74 mg/L (56%)
Mysid (*Americamysis bahia*)			
Acute (96-h LC50)			3.83 mg/L (78.2%)
Chronic (Survival)		NOEC	2.74 mg/L (56%)
		LOEC	4.90 mg/L (100%)
Chronic (Growth)	NOEC		2.74 mg/L (56%)
		LOEC	4.90 mg/L (100%)
Chronic (Fecundity)		NOEC	2.74 mg/L (56%)
		LOEC	4.90 mg/L (100%)
Daphnid (*Ceriodaphnia dubia*)			
Acute (48-h LC50)			Not acutely toxic at 9.20 mg/L (100%)
Chronic (Survival)		NOEC	Not chronically toxic at 9.2 mg/L (100%)
Chronic (Reproduction)		NOEC	2.74 mg/L (56%)
		LOEC	9.20 mg/L (100%)
Algae (*Selenastrum capricornutum*)			
		NOEC	Not chronically toxic at 9.2 mg/L (100%)
Amphipod *Hyalella azteca* 10-d (survival)			Not acutely toxic at 2.94 mg/L (32%)[2]
Amphipod *Hyalella azteca* 28-d (survival, growth, reproduction)			Not acutely toxic at 2.94 mg/L (32%)
Amphipod *Leptocheirus plumulosus* 10-d (survival)			Not acutely toxic at 1.57 mg/L (32%)
Amphipod *Leptocheirus plumulosus* 28-d (survival, growth, reproduction)			Not acutely toxic at 1.57 mg/L (32%)

[1]Water column toxicity expressed as % stock solution by volume based on total organic carbon
[2]Sediment toxicity expressed as percent compound in sediment by weight

Table 7: Test results for BAK 2195/CP1000 (90:10) water column and sediment toxicity tests

Species - Test – Endpoint			BAK 2195/CP1000 (90:10)
Fathead minnow (*Pimephales promelas*)			
	Acute (96-h LC50)		Not acutely toxic at 18.00 mg/L (100%)[1]
	Chronic (Survival)	NOEC	Not toxic at 18.00 mg/L (100%)
	Chronic (Growth)	NOEC	Not toxic at 18.00 mg/L (100%)
Sheepshead minnow (*Cyprinodon variegatus*)			
	Acute (96-h LC50)		Not acutely toxic at 17.80 mg/L (100%)
	Chronic (Survival)	NOEC	Not toxic at 17.80 mg/L (100%)
	Chronic (Growth)	NOEC	Not toxic at 17.80 mg/L (100%)
Mysid (*Americamysis bahia*)			
	Acute (96-h LC50)		Not toxic at 17.80 mg/L (100%)
	Chronic (Survival)	NOEC	Not toxic at 17.80 mg/L (100%)
	Chronic (Growth)	NOEC	Not toxic at 17.80 mg/L (100%)
	Chronic (Fecundity)	NOEC	Not toxic at 17.80 mg/L (100%)
Daphnid (*Ceriodaphnia dubia*)			
	Acute (48-h LC50)		Not toxic at 18.00 mg/L (100%)
	Chronic (Survival)	NOEC	Not toxic at 18.00 mg/L (100%)
	Chronic (Reproduction)	NOEC	Not toxic at 18.00 mg/L (100%)
Algae (*Selenastrum capricornutum*)			
		NOEC	Not toxic at 18.00 mg/L (100%)
Amphipod *Hyalella azteca* 10-d (survival)			Not toxic at 18.00 mg/L (100%)[2]
Amphipod *Hyalella azteca* 28-d (survival, growth, reproduction)			Not toxic at 18.00 mg/L (100%)
Amphipod *Leptocheirus plumulosus* 10-d (survival)			Not toxic at 17.80 mg/L (100%)
Amphipod *Leptocheirus plumulosus* 28-d (survival, growth, reproduction)			Not toxic at 17.80 mg/L (100%)

[1]Water column toxicity expressed as % stock solution by volume based on total organic carbon
[2]Sediment toxicity expressed as percent compound in sediment by weight

Table 8: Test results for BAK 402 - 005 water column and sediment toxicity tests

Species - Test – Endpoint	BAK402 - 005
Fathead minnow (*Pimephales promelas*)	
Acute (96-h LC50)	Not acutely toxic at 731.30 mg/L (100%)[1]
Chronic (Survival) NOEC	Not toxic at 731.30 mg/L (100%)
Chronic (Growth) NOEC	Not toxic at 731.30 mg/L (100%)
Sheepshead minnow (*Cyprinodon variegatus*)	
Acute (96-h LC50)	Not acutely toxic at 19.10 mg/L (100%)[1]
Chronic (Survival) NOEC	Not toxic at 19.10 mg/L (100%)
Chronic (Growth) NOEC	Not toxic at 19.10 mg/L (100%)
Mysid (*Americamysis bahia*)	
Acute (96-h LC50)	Not acutely toxic at 19.10 mg/L (100%)[1]
Chronic (Survival) NOEC	Not toxic at 19.10 mg/L (100%)
Chronic (Growth) NOEC	10.70 mg/L (56%)
Chronic (Fecundity) NOEC	10.70 mg/L (56%)
Daphnid (*Ceriodaphnia dubia*)	
Acute (48-h LC50)	Not acutely toxic at 731.30 mg/L (100%)[1]
Chronic (Survival) NOEC	Not toxic at 731.30 mg/L (100%)
Chronic (Reproduction) NOEC	Not toxic at 731.30 mg/L (100%)
Algae (*Selenastrum capricornutum*)	
NOEC	409.53 mg/L (56%)
LOEC	731.30 mg/L (100%)
Amphipod *Hyalella azteca* 10-d (survival)	Not toxic at 409.52 mg/L (56%)[2]
Amphipod *Hyalella azteca* 28-d (survival, growth, reproduction)	Not toxic at 409.52 mg/L (56%)
Amphipod *Leptocheirus plumulosus* 10-d (survival)	Not toxic at 10.70 mg/L (56%)
Amphipod *Leptocheirus plumulosus* 28-d (survival, growth, reproduction)	Not toxic at 10.70 mg/L (56%)

[1]Water column toxicity expressed as % stock solution by volume based on total organic carbon
[2]Sediment toxicity expressed as percent compound in sediment by weight

Table 9: Test results for PVA CP1000 water column and sediment toxicity tests

Species - Test – Endpoint			PVA CP1000
Fathead minnow (*Pimephales promelas*)			
Acute (96-h LC50)			Not acutely toxic at 308.30 mg/L (100%)[1]
Chronic (Survival)	NOEC		Not toxic at 308.30 mg/L (100%)
Chronic (Growth)	NOEC		Not toxic at 308.30 mg/L (100%)
Sheepshead minnow (*Cyprinodon variegatus*)			
Acute (96-h LC50)			Not acutely toxic at 39.00 mg/L (100%)[1]
Chronic (Survival)	NOEC		Not toxic at 39.00 mg/L (100%)
Chronic (Growth)	NOEC		Not toxic at 39.00 mg/L (100%)
Mysid (*Americamysis bahia*)			
Acute (96-h LC50)			14.31 mg/L (36.7%)
Chronic (Survival)	NOEC		3.90 mg/L (10%)
		LOEC	7.02 mg/L (18%)
Chronic (Growth)	NOEC		2.18 mg/L (5.6%)
		LOEC	3.90 mg/L (10%)
Chronic (Fecundity)		NOEC	2.18 mg/L (5.6%)
		LOEC	3.90 mg/L (10%)
Daphnid (*Ceriodaphnia dubia*)			
Acute (48-h LC50)			238.32 mg/L (77.3%)
Chronic (Survival)		NOEC	98.66 mg/L (32%)
		LOEC	172.64 mg/L (56%)
Chronic (Reproduction)		NOEC	5.55 mg/L (1.8%)
		LOEC	9.87 mg/L (3.2%)
Algae (*Selenastrum capricornutum*)			
		NOEC	172.64 mg/L (56%)
		LOEC	308.30 mg/L (100%)
Amphipod *Hyalella azteca* 10-d (survival)			Not toxic at 5.55 mg/L (1.8%)
Amphipod *Hyalella azteca* 28-d (survival, growth, reproduction)			Not toxic at 5.55 mg/L (1.8%)
Amphipod *Leptocheirus plumulosus* 10-d (survival)			Not toxic at 0.70 mg/L (1.8%)
Amphipod *Leptocheirus plumulosus* 28-d (survival, growth, reproduction)			Not toxic at 0.70 mg/L (1.8%)

[1]Water column toxicity expressed as % stock solution by volume based on total organic carbon
[2]Sediment toxicity expressed as percent compound in sediment by weight

Table 10: Percent emergence tests: *B. rappa*			
Treatment	Average	SE	ANOVA
Negative control	9.2	0.2	
Boric acid: 40 ppm	8.8*	0.4	
Boric acid: 80 ppm	7.0*,++	0.6	$p<0.0001$
Boric acid: 160 ppm	2.8*,++	0.5	
Boric acid: 320 ppm	0.0*,++	-	
Polytriticum 2000: 2.90 ppm	9.5	0.5	
Polytriticum 2000: 5.79 ppm	9.5	0.5	
Polytriticum 2000: 11.59 ppm	7.0*	-	
Polytriticum 2000: 24.14 ppm	8.0	1.0	
Polytriticum 2000: 72.42 ppm	9.0	1.0	
Polytriticum 2000: 96.56 ppm	9.0	0.5	
PVA CP1000: 3,000 ppm	8.5	1.5	
PVA CP1000: 12,500 ppm	8.5	0.5	
PVA CP1000: 18,750 ppm	9.5	0.5	
PVA CP1000: 25,000 ppm	9.0	1.0	
BIOPOL D411GN: 0.6 ppm	9.0	1.0	
BIOPOL D411GN: 2.5 ppm	10.0	-	
BIOPOL D411GN: 3.8 ppm	7.0*	1.0	
BIOPOL D411GN: 5.0 ppm	9.5	0.5	
BAK 402-005: 40.3 ppm	9.5	0.5	
BAK 402-005: 80.6 ppm	10.0	-	
BAK2195/CP1000 (90:10): 20.12 ppm	9.5	0.5	
BAK2195/CP1000 (90:10): 40.23 ppm	9.5	0.5	

*Difference is statistically significant by the Fisher's Exact Test at the $p<0.05$ level when compared with negative controls

++ Difference is statistically significant by the Bonferroni/Dunn Test at the $p<0.002$ level when compared with negative controls

Table 11: Average shoot length at emergence: *B. rappa*			
Treatment	Average (cm)	SE	ANOVA
Negative control	2.0	0.1	
Boric acid: 40 ppm	1.7*	0.1	
Boric acid: 80 ppm	1.0*,++	0.1	$p<0.0001$
Boric acid: 160 ppm	0.4*,++	-	
Boric acid: 320 ppm	0.2*,++	-	
Polytriticum 2000: 2.90 ppm	1.9	0.1	
Polytriticum 2000: 5.79 ppm	2.2	0.1	
Polytriticum 2000: 11.59 ppm	2.2	0.1	
Polytriticum 2000: 24.14 ppm	2.3	0.2	
Polytriticum 2000: 72.42 ppm	1.9	0.2	
Polytriticum 2000: 96.56 ppm	1.7	0.1	
PVA CP1000: 3,000 ppm	2.1	0.2	
PVA CP1000: 12,500 ppm	2.0	0.2	
PVA CP1000: 18,750 ppm	2.1	0.1	
PVA CP1000: 25,000 ppm	2.1	0.2	
BIOPOL D411GN: 0.6 ppm	1.9	0.2	
BIOPOL D411GN: 2.5 ppm	2.1	0.1	
BIOPOL D411GN: 3.8 ppm	1.8	0.1	
BIOPOL D411GN: 5.0 ppm	1.7*	0.1	
BAK 402-005: 40.3 ppm	2.0	0.1	
BAK 402-005: 80.6 ppm	2.2	0.2	
BAK2195/CP1000 (90:10): 20.12 ppm	1.5*	0.1	
BAK2195/CP1000 (90:10): 40.23 ppm	1.3*,++	0.1	

*Difference is statistically significant by the Fisher's Exact Test at the $p<0.05$ level when compared with negative controls

++ Difference is statistically significant by the Bonferroni/Dunn Test at the $p<0.002$ level when compared with negative controls

Table 12: Percent emergence tests: Watercress			
Treatment	Average	SE	ANOVA
Negative control	7.6	1.7	$p<0.0001$
Boric acid: 40 ppm	9.4*	0.6	
Boric acid: 80 ppm	8.6	1.5	
Boric acid: 160 ppm	5.8*	1.1	
Boric acid: 320 ppm	2.0*,++	-	
Polytriticum 2000: 11.59 ppm	9.0	-	
Polytriticum 2000: 72.42 ppm	10.0*	-	
Polytriticum 2000: 96.56 ppm	9.0	-	
PVA CP1000: 3,000 ppm	9.0	-	
PVA CP1000: 12,500 ppm	8.0	-	
PVA CP1000: 18,750 ppm	8.0	2.8	
PVA CP1000: 25,000 ppm	8.0	-	
BIOPOL D411GN: 0.6 ppm	9.0	1.4	
BIOPOL D411GN: 2.5 ppm	10.0*	-	
BIOPOL D411GN: 3.8 ppm	7.0	1.4	
BIOPOL D411GN: 5.0 ppm	9.5	0.7	
BAK 402-005: 40.3 ppm	9.5	0.7	
BAK 402-005: 80.6 ppm	8.5	0.7	
BAK2195/CP1000 (90:10): 20.12 ppm	9.0	-	
BAK2195/CP1000 (90:10): 40.23 ppm	8.5	0.7	

*Difference is statistically significant by the Fisher's Exact Test at the $p<0.05$ level when compared with negative controls

++ Difference is statistically significant by the Bonferroni/Dunn Test at the $p<0.002$ level when compared with negative controls

Table 13: Average shoot length at emergence: Watercress			
Treatment	Average (cm)	SE	ANOVA
Negative control	2.4	0.1	$p<0.0001$
Boric acid: 40 ppm	2.1*	0.1	
Boric acid: 80 ppm	1.4*,++	0.1	
Boric acid: 160 ppm	0.6*,++	0.1	
Boric acid: 320 ppm	0.4*,++	0.1	
Polytriticum 2000: 11.59 ppm	2.6*	0.2	
Polytriticum 2000: 24.14 ppm	3.2*,++	0.1	
Polytriticum 2000: 72.42 ppm	3.0*,++	0.2	
Polytriticum 2000: 96.56 ppm	2.8*	0.2	
PVA CP1000: 3,000 ppm	2.8*	0.1	
PVA CP1000:12,500 ppm	2.9*	0.1	
PVA CP1000:18,750 ppm	2.3	0.1	
PVA CP1000:25,000 ppm	2.1	0.1	
BIOPOL D411GN: 0.6 ppm	2.6	0.2	
BIOPOL D411GN: 2.5 ppm	2.5	0.2	
BIOPOL D411GN: 3.8 ppm	2.6	0.2	
BIOPOL D411GN: 5.0 ppm	2.7	0.2	
BAK402-005:40.3 ppm	2.9*	0.1	
BAK402-005:80.6 ppm	2.2	0.2	
BAK2195/CP1000 (90:10): 20.12 ppm	1.8*	0.2	
BAK2195/CP1000 (90:10): 40.23 ppm	2.0*	0.1	

*Difference is statistically significant by the Fisher's Exact Test at the $p<0.05$ level when compared with negative controls

++ Difference is statistically significant by the Bonferroni/Dunn Test at the $p<0.002$ level when compared with negative controls

Table 14: Aquatic toxicity values for common chemicals[1]		
Chemical	Species	Result (mg/L)
Copper	Fathead minnow (96 h LC50)	0.3 - 21.0
	Fathead minnow (7 day chronic)	0.05 – 1.1
	Ceriodaphnia (48 h LC50)	0.01-1.0
	Sheepshead minnow (96 h LC50)	0.4
	Mysid (96 h LC50)	0.08 – 0.25
	Hyallella azteca (10-d mortality)	0.02–0.16
	Hyallella azteca (28-d mortality)	0.02-0.03
Ammonia	Fathead minnow (96 h LC50)	5.9 – 8.2
	Ceriodaphnia (48 h LC50)	2.0-3.7
	Sheepshead minnow (96 h LC50)	1.9-3.6
Nitrite	Fathead minnow (96 h LC50)	2.3 - 3.0
Sodium chloride	Fathead minnow (96 h LC50)	6,020 - 10,000
	Ceriodaphnia (48 h LC50)	1,170 – 3,540
Ethanol	Fathead minnow (96 h LC50)	13,000 – 18,000
	Ceriodaphnia (48 h LC50)	1,284 – 12,000
[1]Source: USEPA ECOTOX Database System		

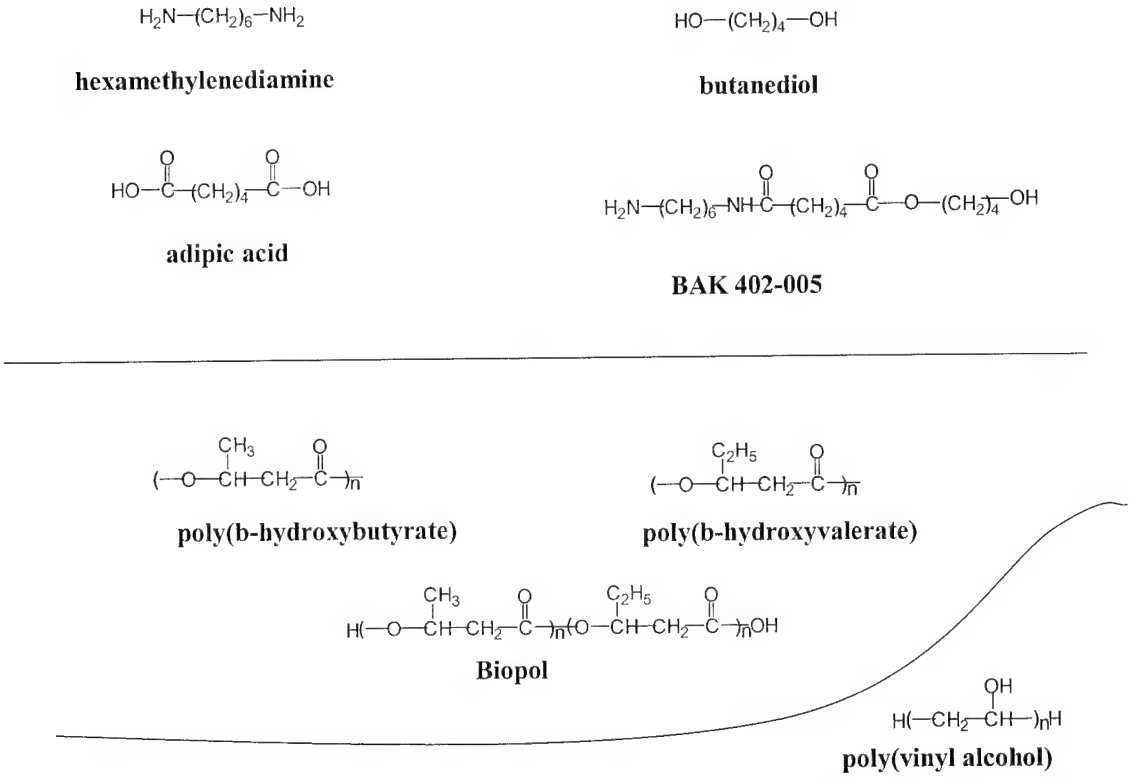

Figure 1: Chemical structures of the biopolymers and several of their precursors

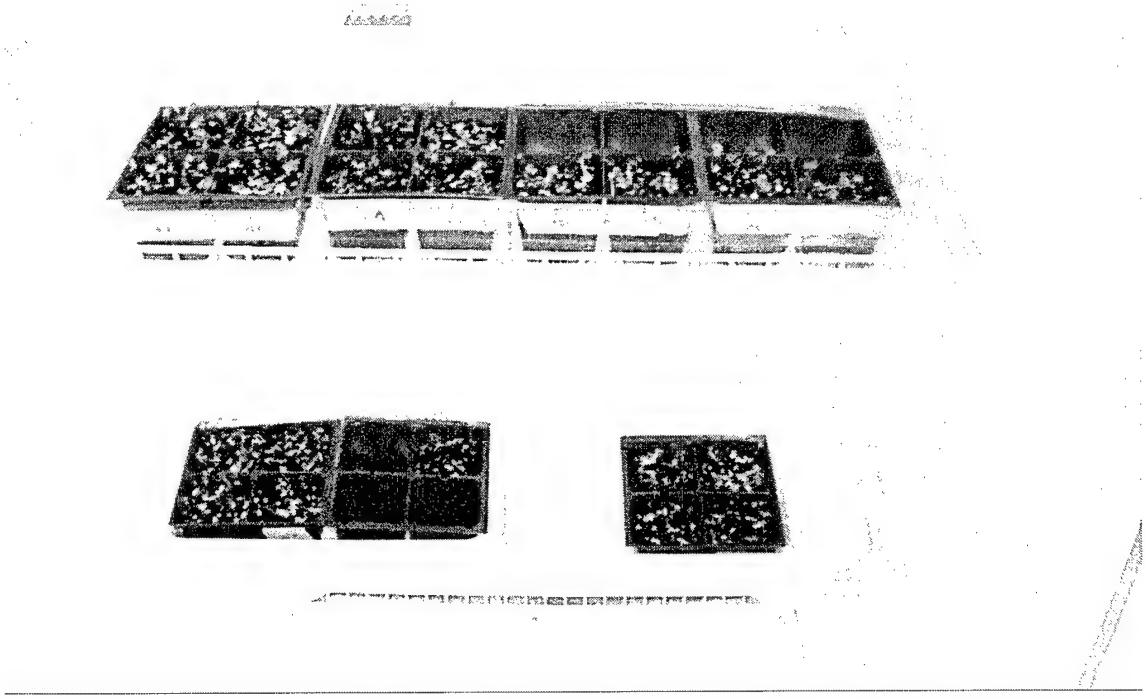

Figure 2: Emergence and elongation tests being carried out in a low illumination incubator

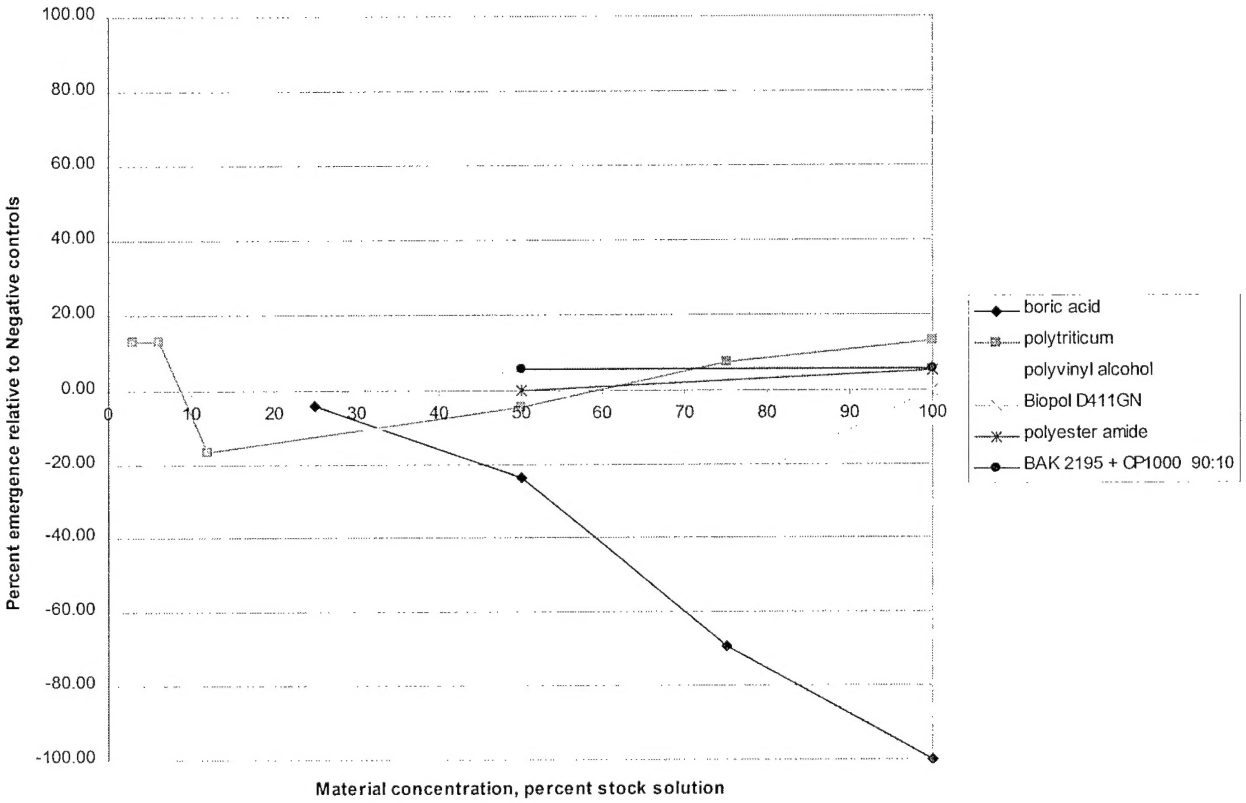

Figure 3: Percent emergence of *B. rappa* seeds expressed as a percentage of the mean number of negative control seeds that emerged during the 6 day study period

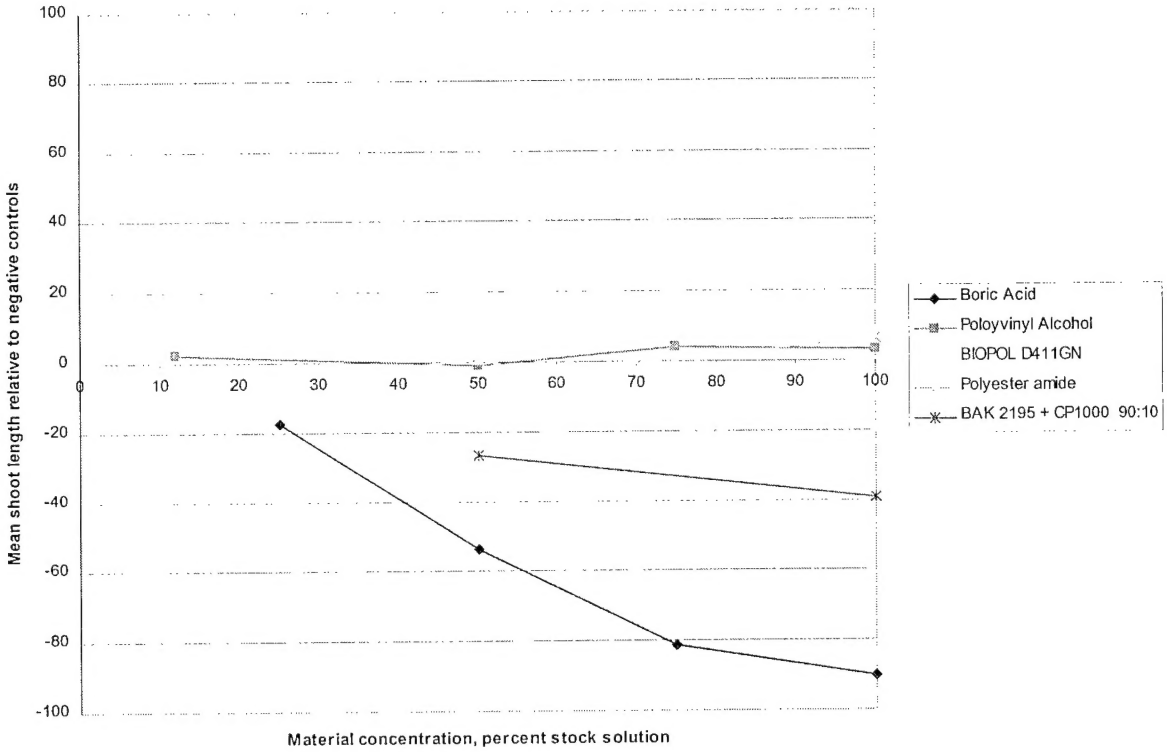

Figure 4: Percent difference in average shoot length of emergent treated versus control *B. rappa*

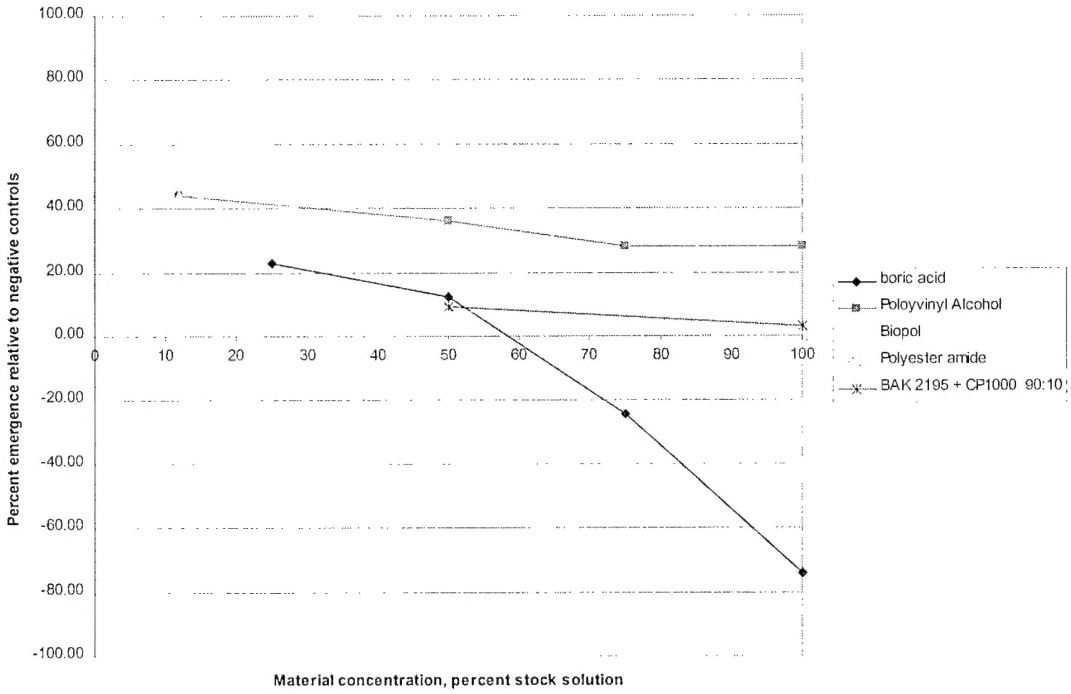

Figure 5: Percent emergence of watercress seeds expressed as a percentage of the mean number of negative control seeds that emerged during the 6 day study period

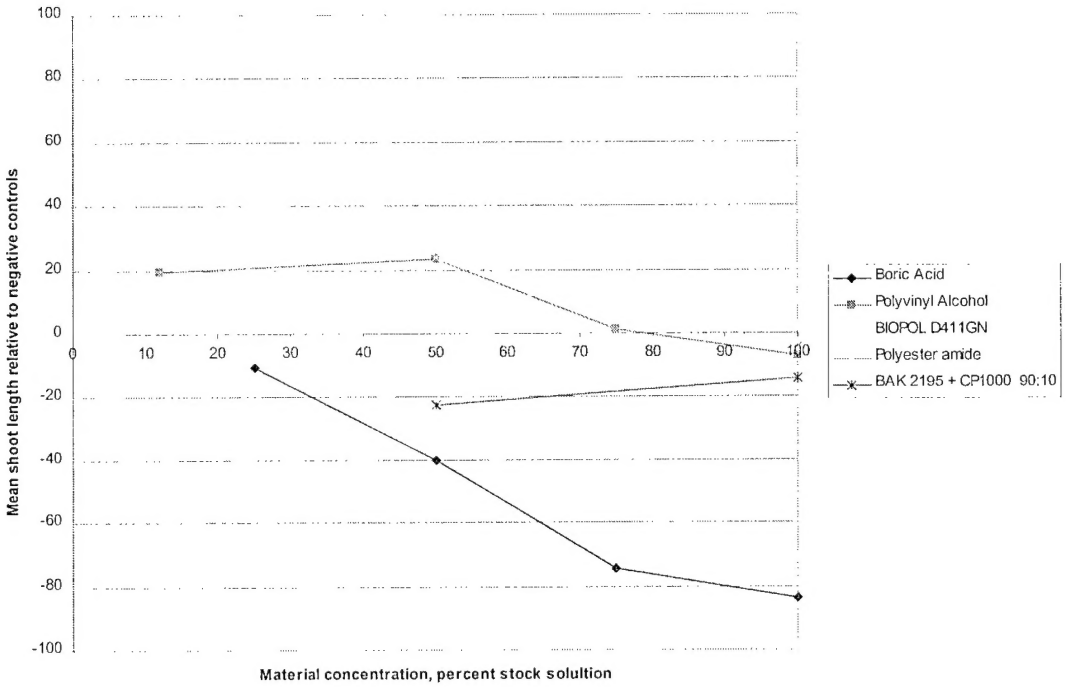

Figure 6: Percent difference in average shoot length of emergent treated versus control watercress